

University of Groningen

## Symptomatic and asymptomatic airway hyperresponsiveness

Jansen, Désirée Frederique

**IMPORTANT NOTE:** You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

*Document Version*

Publisher's PDF, also known as Version of record

*Publication date:*

1999

[Link to publication in University of Groningen/UMCG research database](#)

*Citation for published version (APA):*

Jansen, D. F. (1999). *Symptomatic and asymptomatic airway hyperresponsiveness: epidemiological and pathological studies*. [Thesis fully internal (DIV), University of Groningen]. [s.n.].

### Copyright

Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

The publication may also be distributed here under the terms of Article 25fa of the Dutch Copyright Act, indicated by the "Taverne" license. More information can be found on the University of Groningen website: <https://www.rug.nl/library/open-access/self-archiving-pure/taverne-amendment>.

### Take-down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Downloaded from the University of Groningen/UMCG research database (Pure): <http://www.rug.nl/research/portal>. For technical reasons the number of authors shown on this cover page is limited to 10 maximum.

## Chapter 7

---

### **SUMMARY, GENERAL DISCUSSION, CONCLUSIONS AND PERSPECTIVES**

---

## Summary and general discussion

In this thesis, we described the relationship of various allergy markers and the development of pulmonary function with the single or coinciding presence of respiratory symptoms and airway hyperresponsiveness (AHR) in a general adult population. Further, we investigated whether differences in inflammatory cells exist in the airways of subjects with and without respiratory symptoms and AHR. To study these relationships, we analyzed already existing data from the Vlagtwedde-Vlaardingen Study from 1965 to 1990, and we collected new data in a subsample of subjects who participated in one of the final surveys at Vlagtwedde in 1985 or 1989. *Chapter 1* includes the background of this study, together with a more detailed description of the Vlagtwedde-Vlaardingen Study. Further, factors associated with airway hyperresponsiveness (AHR) and possible mechanisms of AHR are discussed.

### ***Considerations on the study population and methods***

In 1995 and 1996, we invited a subsample of participants of the Vlagtwedde Survey of 1989 or 1985, because asymptomatic individuals with airway hyperresponsiveness are hard to find in the general population and this allowed us to include subjects more efficiently. We invited all participants who experienced respiratory symptoms with or without AHR (Sy+AHR+, Sy+AHR-) at the final survey in 1989 or 1985, a random sample of subjects who were asymptomatic without AHR (Sy-AHR-), and a sample of asymptomatic subjects with AHR (Sy-AHR+). The asymptomatic subjects with more severe AHR were invited first for evaluation to increase the chance that they were still hyperresponsive at the current testing. This means that the new study was not performed in a random sample of subjects participating the final surveys of Vlagtwedde, and the four groups under study (Sy-AHR-, Sy-AHR+, Sy+AHR-, Sy+AHR+) may not be representative for the same groups in the general population due to selection bias. To determine whether selection bias influenced our results, we compared the characteristics of each group with the characteristics of the same group in 1989 (excluding the subjects who participated in the study of 1995/1996). Mean age was significantly higher in the Sy-AHR- group in 1995/96 than in the Sy-AHR- group in 1989 (Table 1). The levels of FEV<sub>1</sub> % predicted were significantly higher in Sy-AHR- and Sy-AHR+ subjects in 1995/96 than in these groups evaluated in 1989. Further, the prevalence of skin test positivity was higher in 1995/96 than in 1989 in all groups. A more sensitive method in 1995/96, i.e. intracutaneous skin tests of 12 common aeroallergens in 1995/96, and skin prick tests to 6 common aeroallergens in 1989, may explain the difference in prevalence. This was confirmed by comparison of the prevalence of skin test positivity in 1989 between the subjects who did and did not participate in the new study, which showed no differences within the four groups.

**Table 1. Characteristics of the study population of Vlagtwedde in 1989 and 1995/1996 according to respiratory symptoms (Sy) and airway hyper-responsiveness (AHR)**

	Respiratory symptom and AHR status				Total
	Sy-AHR-	Sy-AHR+	Sy+AHR-	Sy+AHR+	
Characteristics of the study population in 1989 <sup>1</sup>					
Total number (% men)	138 (59.4)	229 (53.3)	14 (35.7)	48 (62.5)	429 (55.7)
Age, yr <sup>2</sup>	50.9±8.9	53.8±9.0	55.2±9.6	55.3±7.9	53.1±9.0
Smoking habits, n (%)					
never smoker	50 (37.3)	69 (30.9)	5 (35.7)	15 (31.9)	139 (33.3)
ex-smoker	53 (39.6)	76 (34.1)	5 (35.7)	14 (29.8)	148 (35.4)
current smoker	31 (23.1)	78 (35.0)	4 (28.6)	18 (38.3)	131 (31.3)
FEV <sub>1</sub> , %pred. <sup>2</sup>	114.5±14.4	104.8±13.9	109.8±9.9	102.2±20.7	107.8±15.6
Eosinophilia, n (%)	20 (14.5)	41 (18.3)	1 (7.7)	6 (12.5)	68 (16.1)
Skin test positive, n (%)	13 (10.2)	23 (11.4)	1 (7.1)	4 (9.1)	41 (10.6)
Characteristics of the study population in 1995/1996					
Total number (% men)	19 (63.2)	12 (41.7)	12 (58.3)	14 (50.0)	57 (54.4)
Age, yr <sup>2</sup>	56.3±7.4*	54.3±4.8	54.5±7.4	58.6±10.0	56.1±7.7
Smoking habits, n (%)					
never smoker	7 (36.8)	3 (25.0)	2 (16.7)	1 (7.1)	13 (22.8)
ex-smoker	10 (52.6)	7 (58.3)	7 (58.3)	6 (42.9)	30 (52.6)
current smoker	2 (10.5)	2 (16.7)	3 (25.0)	7 (50.0)	14 (24.6)
FEV <sub>1</sub> , %pred. <sup>2</sup>	124.8±19.3*	113.4±15.9*	118.0±16.4	102.2±20.7	115.4±19.9
Eosinophilia, n (%)	1 (5.3)	4 (33.3)	1 (8.3)	5 (35.7)	11 (19.3)
Skin test positive, n (%)	7 (36.8)*	5 (41.7)*	5 (41.7) <sup>†</sup>	4 (28.6) <sup>‡</sup>	21 (36.8)

<sup>1</sup> excluding the subjects who participated the study in 1995/1996; <sup>2</sup> Age and FEV<sub>1</sub> % pred. are expressed as mean ± standard deviation; \* p < 0.05 compared with the same group in 1989, <sup>†</sup> p = 0.065 compared with Sy+AHR- subjects in 1989, <sup>‡</sup> p = 0.086 compared with Sy+AHR+ subjects in 1989. Differences in characteristics were not tested between the total group of 1989 and 1995/96, as the proportional distribution of subjects between the groups differ.

The Vlagtwedde-Vlaardingen Study is one of the world's longest-lasting (1965 to 1990) and most extensive longitudinal population studies. Presently, high costs of research investigations and increased mobility of inhabitants make it almost impossible to start a study like this. A great advantage of longitudinal studies when using the same methods at subsequent surveys in the same individuals, is the opportunity to study changes within a person. In our population, the same questionnaire was used at each survey to determine the prevalence of respiratory symptoms and smoking habits. A change in symptom status, and thus the incidence and remission of respiratory symptoms, and in smoking habits could be determined in this way. Further, pulmonary function and airway responsiveness were assessed in the same way each survey. Thus, to retain the comparability between surveys within this 25-year follow-up study the same methods were used in all surveys. A small drawback is that this sometimes leads to a more difficult comparison with more recent studies that use new methods. This was the case for skin tests and airway hyperresponsiveness. The Vlagtwedde-Vlaardingen Study was one of the first longitudinal population studies that measured skin test positivity and airway responsiveness. Skin tests were performed at the baseline surveys (1965, 1967/1969), the first follow-up surveys (1970/1972) and the final surveys (1989/1990). At the final surveys standardized allergens were used, whereas these were not yet available at the time of the baseline surveys in the sixties and the first follow-up surveys. To determine airway hyperresponsiveness, De Vries and coworkers modified the method of Tiffeneau (1) according to the standardization guidelines (2) into 30 seconds of inhalation of doubling doses of histamine solutions. In contrast to current practice in research, a consistent 10% decrease in  $FEV_1$  or IVC was used to determine airway responsiveness (expressed as a  $PC_{10}$  value). A subject with a  $PC_{10}$  value of  $\leq 16$  mg/ml histamine was considered to be hyperresponsive. Nowadays, a 2 minutes inhalation method is commonly used and airway hyperresponsiveness is defined as a decrease in  $FEV_1$  of at least 20% at a concentration of  $\leq 8$  mg/ml histamine or methacholine ( $PC_{20}$  value). Recent studies generally prefer to use methacholine instead of histamine as stimulus to determine airway responsiveness, because of detrimental side effects at higher concentrations of histamine. Although different stimuli are used, both stimuli act directly on smooth muscle cells and airway responsiveness to histamine and methacholine are comparable (3,4). Previous results comparing various indices of airway responsiveness have shown comparability between a  $PC_{10} \leq 16$  mg/ml and a  $PC_{20} \leq 8$  mg/ml (5). Another difference with recent studies is the definition of AHR based on a decrease in  $FEV_1$  or IVC. Only a small number of subjects were considered hyperresponsive solely on the basis of a consistent 10% decrease in IVC. In the final surveys, however, more than 30% of the hyperresponsive subjects appeared to be categorized this way solely on the basis of a decrease in IVC. Since after each inhalation both  $FEV_1$  and IVC were measured twice, tiredness of the study subjects may have influenced the outcome. To reduce the likelihood of misclassification of AHR- subjects as AHR+ subjects, we lowered the threshold value and used a more stringent definition of AHR ( $PC_{10} \leq 8$  mg/ml).

In 1995 and 1996, we collected new data on a sample of subjects who participated in one of the final surveys at Vlagtwedde in 1985 or 1989. Although we used as many methods as possible similar to those used in the Vlagtwedde-Vlaardingen Study, including the questionnaire and pulmonary function measurements, some changes were made. This was necessary in order to make it possible to compare our results with results of other recent studies. Airway responsiveness was determined by 2 minutes inhalation instead of 30 seconds and AHR was defined as a consistent 20% decrease in  $FEV_1$  at a concentration of  $\leq 8$  mg/ml histamine ( $PC_{20} \leq 8$  mg/ml). Further, skin test positivity was determined intracutaneously using standardized allergens. Another difference with the previous longitudinal surveys was that individual measurements were performed at the University Hospital Groningen throughout the year. The longitudinal surveys were all performed at a community building in the area, during two weeks in the month of October. The use of different methods should caution comparison of these results with the former results of the longitudinal study and may affect the interpretation of changes within the subjects from 1985 or 1989 to 1995/1996.

In addition to spirometry, histamine challenge testing, and measurement of peripheral blood eosinophils, biopsies were taken to provide direct information on the situation in the airway wall where the interaction between the environmental and host factors takes place. Fiberoptic bronchoscopy was performed according to the guidelines of the American Thoracic Society (6). Biopsies of normal appearing mucosa were taken from the subcarinae of the right lower or middle lobe, using a fenestrated forceps (FB-21C, Olympus, Tokyo, Japan).

In *Chapter 2* an overview of the existing literature was given on factors associated with AHR, which may explain the presence of AHR in both symptomatic and asymptomatic individuals. Although AHR is generally accompanied by respiratory symptoms, the prevalence of AHR in asymptomatic subjects in the general population ranges from 2 to 14%. Several factors have been put forward to explain this phenomenon. First, the use of a definition for AHR that is not stringent enough. There is, however, no cut-off point of AHR to distinguish completely symptomatic from asymptomatic subjects. Second, viral respiratory infections, occupational sensitizers, or allergen exposure may be responsible for a temporal change in airway responsiveness in asymptomatic subjects. Third, asymptomatic subjects may not recognize variable airways obstruction as breathlessness. Finally, the presence of respiratory symptoms may modify the relation of certain risk factors with AHR. The relation of older age, atopy, and parental smoking with AHR appeared to be stronger in symptomatic subjects than asymptomatic subjects, whereas in contrast, a family history of asthma only related to AHR in asymptomatic subjects.

An important finding described in the literature is that asymptomatic subjects with AHR are more prone to develop respiratory symptoms than asymptomatic subjects without AHR. Even in the absence of respiratory symptoms, it is likely that inflammatory changes in the airways play an important role as one of the underlying mechanisms for AHR in asymptomatic subjects. Current studies in asthma suggest

that an intricate interaction between different types of inflammatory cells and their respective mediators play a role in the processes that lead to increased airway responsiveness. Only a few studies have examined the presence of inflammatory changes in asymptomatic subjects with AHR. Although the number of eosinophils in peripheral blood was found to be increased in asymptomatic subjects with AHR compared with asymptomatic subjects without AHR, AHR in asymptomatic subjects was not found to be associated with overall inflammatory changes in the airways. This may be the case in an asymptomatic state, yet one study did suggest that progression of an asymptomatic to a symptomatic state is associated with increasing inflammation. Furthermore, it is well possible that the reaction to inhaled irritants and viral infections is different in asymptomatic individuals with and without AHR, thereby ultimately leading to a different clinical outcome.

In *Chapter 3* the independent relationship of airway hyperresponsiveness with three different parameters of atopy, i.e. skin test positivity, peripheral blood eosinophilia, and a high serum total IgE level, has been described. Since we were interested in differences in mechanisms underlying asymptomatic AHR and symptomatic AHR in particular, we also determined these relationships in subjects with and without respiratory symptoms. Logistic regression analyses were performed using data of 620 subjects who participated the Vlagtwedde-Vlaardingen Study of 1989 and 1990. All analyses were adjusted for age, sex, smoking habits, and urban area of residence. Peripheral blood eosinophilia and skin test positivity were significantly associated with the presence of AHR, whereas a serum total IgE level > 100 kU/L was not significantly associated with AHR. Separate analyses for symptomatic and asymptomatic subjects showed that the higher risk of AHR with skin test positivity applied only to symptomatic subjects, independent of peripheral blood eosinophilia and high serum total IgE levels. In contrast, the higher risk of AHR with peripheral blood eosinophilia was present in both symptomatic and asymptomatic subjects, independent of skin test positivity and high serum total IgE levels. The results of this study show that, in a general adult population, symptomatic and asymptomatic individuals with peripheral blood eosinophilia are more likely to have AHR than individuals without peripheral blood eosinophilia, whereas individuals with skin test positivity are more likely to have AHR only when respiratory symptoms are present. In contrast, high serum total IgE levels were not associated with AHR in this middle-aged population.

Previous analyses using the Vlagtwedde-Vlaardingen data, already showed an increased risk for developing respiratory symptoms in hyperresponsive subjects (7). Since both peripheral blood eosinophilia and skin test positivity are related to the presence of respiratory symptoms and AHR, we assessed whether these atopy-related factors and smoking are related to the development of symptoms and whether they influence the increased risk for developing respiratory symptoms in hyperresponsive subjects (*Chapter 4*). To this aim, we analyzed data of the longitudinal Vlagtwedde-Vlaardingen Study (1965 to 1990) using logistic regression analyses with paired observations, taking multiple measurements within a person into

account. In total, 995 men and 792 women contributed 4,403 paired observations. Cigarette smoking was associated with the development of respiratory symptoms in a dose-dependent way. Hyperresponsive individuals were more likely to develop chronic cough and/or phlegm, and bronchitis episodes than individuals without AHR, independent of the presence of peripheral blood eosinophilia. In contrast, the risk of developing wheeze and dyspnea was only increased in hyperresponsive subjects with peripheral blood eosinophilia, and not in subjects with either AHR or peripheral blood eosinophilia. Skin test positivity in the past decreased the risk to develop respiratory symptoms, which was similar in subjects with and without AHR. These results suggest that hyperresponsive subjects whose wheeze and dyspnea symptoms are in remission are likely to develop recurrent respiratory symptoms, especially when peripheral blood eosinophilia is present.

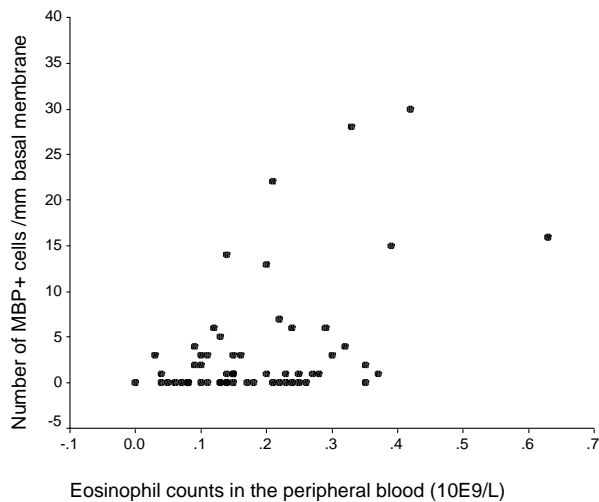
In *Chapter 5* the relationships of various parameters of lung function development (maximally attained level of  $FEV_1$  between the ages 20 and 25, decline in  $FEV_1$  from age 25 onwards, pre-challenge  $FEV_1$  %predicted, and pre-challenge  $FEV_1$  %VC) with the single or coinciding presence of respiratory symptoms and AHR at the final surveys were described. The presence or absence of respiratory symptoms and AHR was known of 691 subjects, aged 35 to 79 yr, who participated in the Vlagtwedde-Vlaardingen Study in 1989 and 1990. The preceding decline in  $FEV_1$  (ml/yr) was calculated by individual regression coefficients including all available  $FEV_1$  measurements from age 25 onwards. Multiple regression analyses showed that a lower pre-challenge level of  $FEV_1$  % predicted or  $FEV_1$  % VC, a lower maximally attained level of  $FEV_1$  % predicted between the ages 20 and 25, and a faster preceding decline in  $FEV_1$  were all associated with the presence of AHR in 1989 and 1990. A lower level of  $FEV_1$  % VC was stronger associated with AHR in symptomatic than in asymptomatic subjects. Further, lower pre-challenge levels of  $FEV_1$  % predicted and  $FEV_1$  % VC were associated with the presence of respiratory symptoms in 1989 and 1990, although only in hyperresponsive subjects. A greater decline in  $FEV_1$  from age 25 onwards was associated with the presence of respiratory symptoms at the final surveys only when the maximally attained level of  $FEV_1$  between the ages 20 and 25 was taken into account (OR=1.34, 95% CI=1.04-1.73). This relation was significantly stronger in subjects with AHR (OR=3.17, 95% CI=1.19-8.42) than in subjects without AHR (OR=1.12, 95% CI=0.84-1.49). We concluded that subjects with a lower maximally attained level of  $FEV_1$  between the ages 20 and 25 and a faster preceding decline in  $FEV_1$  from age 25 onwards are more likely to have airway hyperresponsiveness later in life whereas the actual level of lung function and a faster preceding decline in  $FEV_1$  from age 25 onwards determine whether airway hyperresponsiveness will be accompanied by respiratory symptoms.

In bronchial biopsies of 57 subjects who participated in the final surveys of Vlagtwedde in 1989 or 1985, we assessed whether differences in airway inflammation are related to the presence of respiratory symptoms and airway hyperresponsiveness (*Chapter 6*). In bronchial biopsies of symptomatic and



asymptomatic subjects with and without airway hyperresponsiveness no differences were observed in the total number or subpopulations of T-lymphocytes (CD3, CD4, CD8), the number of B lymphocytes (CD20/22), activated T- or B-lymphocytes (CD25), (activated) eosinophils (MBP, EG2), mast cells (AA1), macrophages (CD68), neutrophils (NP57), and the percentage of vessels with expression of vascular adhesion molecules (ICAM-1, VCAM-1, E-selectin). Heterogeneity of the characteristics of the study population, including atopy and smoking habits, may have increased the variability in inflammatory cell numbers within the four groups under study. Therefore, we cannot exclude the possibility of a more subtle role for an inflammatory process in the airways to differentiate between symptomatic and asymptomatic hyperresponsive subjects. An important finding was the higher number of eosinophils in the airway wall of subjects with peripheral blood eosinophilia than in subjects without peripheral blood eosinophilia. We concluded that this may reflect a more attentive immune response ('pre-activation state') with increased susceptibility to certain stimuli that are capable to activate the eosinophils in the airway wall and subsequently cause AHR and respiratory symptoms.

Epithelial changes, possibly as a consequence of cigarette smoking for many years, may contribute to the development of symptoms in individuals without as well as with airways hyperresponsiveness. Since smoking is associated with both respiratory symptoms and AHR, we hypothesized that the expression of various types of relevant cytokeratins (types 7, 8, 10 and 18) and integrins involved in epithelial adhesion to the extracellular matrix ( $\beta$ 1-integrin VLA2 ( $\alpha$ 2-chain), VLA3 ( $\alpha$ 3-chain) and VLA6 ( $\alpha$ 6-chain), would differ between subjects with and without respiratory symptoms and AHR (*Chapter 6a*). Immunohistological studies were performed using bronchial biopsies of 57 subjects with and without respiratory symptoms and AHR. Cytokeratin 8 and VLA2 were present in almost all cells in all subjects. Cytokeratin 7 (CK7) stained mainly ciliated cells, and less clear in basal cells or goblet cells. Areas with squamous metaplasia showed weak or negative CK7 staining. Cytokeratin 10 was absent in all subjects. Cytokeratin 18 was variably present, mainly in non-basal epithelial cells. VLA3 was seen in epithelium of all subjects and VLA6 in 18 subjects in a variable presence and intensity. Presence of VLA3 and VLA6 in the basal membrane was variable, possibly related to changes in the overlying epithelium. Expression of cytokeratins and integrins in the bronchial epithelium showed no significant differences between the four groups under study. The presence of chronic respiratory symptoms during the previous three years and not necessarily at the time of the study, and the heterogeneity of subject characteristics, such as atopy and smoking habits, might imply that more study subjects are needed to reach sufficient power. Further evaluation of extended study groups should reveal whether early epithelial changes (preceding morphological changes) are related to smoking and to the presence or development of respiratory symptoms. The finding of a subtle decrease in presence of CK7 and gain of VLA6 expression in bronchial epithelium seem worth further exploration, in particular in relation to smoking habits.



**Figure 1.** Number of eosinophils in the bronchial mucosa and in the peripheral blood

### ***The role of eosinophils*** (Figure 2)

Additional analyses using information on the 57 subjects who underwent bronchial biopsy showed that the number of eosinophils in the peripheral blood correlated significantly, though weakly, to the number of eosinophils in the airway wall ( $r_s=0.33$ ,  $p=0.01$ ; Figure 1). The results regarding peripheral blood eosinophilia suggest that there is a signal to the bone marrow that recruits eosinophils to the blood which, although maybe in limited numbers, also allows recruitment of the eosinophils from the blood into the airway wall (by endothelial adhesion and chemotactic factors). The signal to the bone marrow responsible for the higher number of peripheral blood eosinophils may also be responsible for the presence of AHR in asymptomatic subjects. It is likely that cytokines, like IL-5, in a balance with other mediators influencing inflammatory events play an important role (8,9). Of interest are our findings of the longitudinal analyses showing that peripheral blood eosinophilia was an important additional factor to predict the development of wheeze and dyspnea in hyperresponsive individuals. Although we could not demonstrate an association of the presence of (non-activated) MBP-positive eosinophils in the airway wall and respiratory symptoms during the previous three years as evaluated in this study, it is tempting to speculate that peripheral blood eosinophilia and related increased numbers of eosinophils in the airway wall create a local basic bronchial microenvironment that easily allows development and persistence of respiratory symptoms upon a specific stimulus.

The presence of AHR and peripheral blood eosinophilia may reflect a genetic predisposition for the development of respiratory symptoms upon stimulation by the appropriate environmental factors (such as allergen exposure and cigarette smoking) (9,10). This is concordant with the fact that asthma and COPD are multifactorial

diseases. In addition, structural changes of the airway wall and of lung tissue may increase airway responsiveness (11,12). In summary, we suggest that the presence of both AHR and peripheral blood eosinophilia in asymptomatic individuals may reflect a more attentive immune response ('pre-activation state') with increased susceptibility to certain stimuli that are capable to activate the eosinophils in the bronchial wall and subsequently cause AHR and respiratory symptoms.

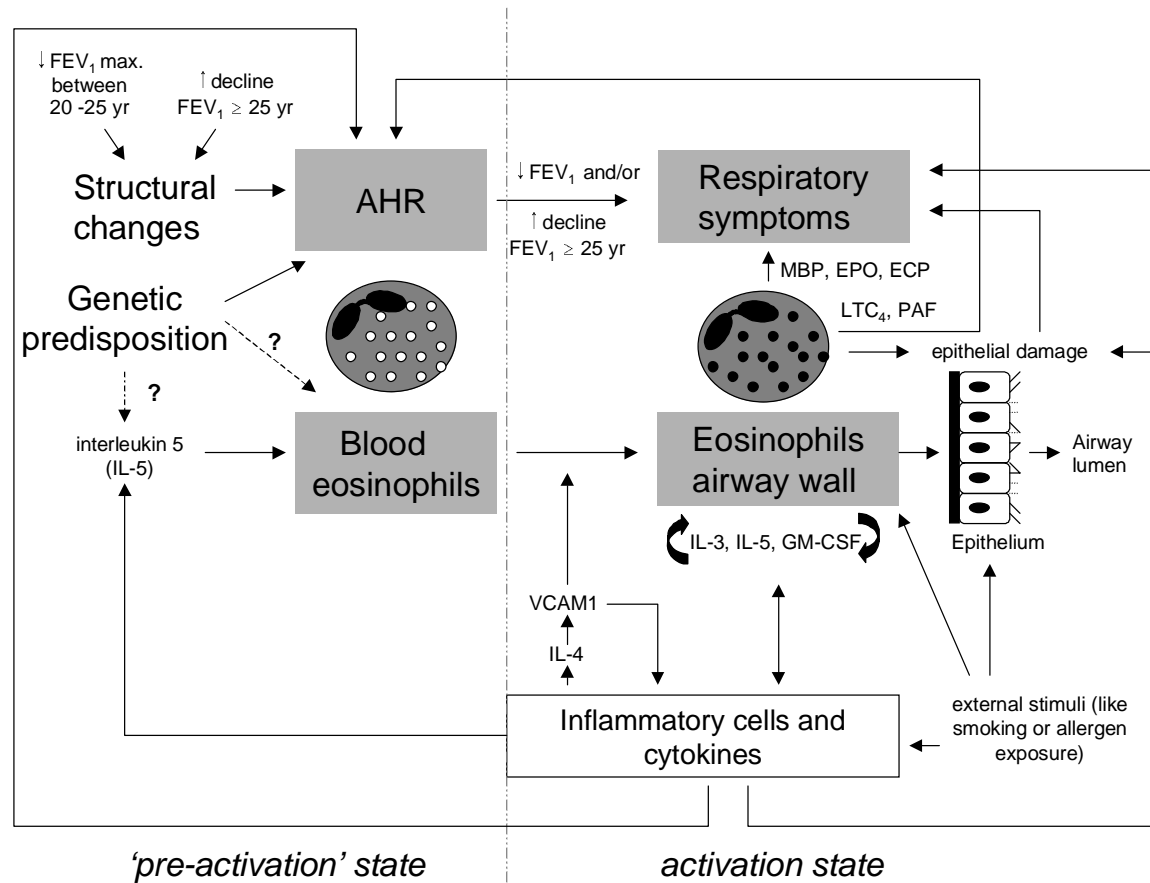
## Conclusions

*With regard to three atopy-related factors in relation to symptomatic and asymptomatic AHR:*

- ◆ Peripheral blood eosinophilia is associated with AHR, independent of the presence of respiratory symptoms, whereas skin test positivity is associated with AHR only in symptomatic subjects (Table 2). A high serum total IgE level ( $> 100$  kU/L) was not associated with AHR in the population under study (Table 2).
- ◆ Although the prevalences of peripheral blood eosinophilia, skin test positivity, and high serum total IgE levels are similar, the overlap among the three atopy-related factors is small. The use of different allergy markers will result in different subjects considered to be atopic, making it difficult to compare the results of studies using these different atopy-related factors.
- ◆ Peripheral blood eosinophilia is strongly associated with AHR especially in subjects who have been intermittently symptomatic, and not in subjects who have never been symptomatic or subjects who have always been symptomatic.
- ◆ Other important factors associated with AHR are an older age and cigarette smoking, in both symptomatic and asymptomatic subjects.

*With regard to the development of respiratory symptoms:*

- ◆ Cigarette smoking increases the risk to develop respiratory symptoms, in a dose-dependent relation.
- ◆ The presence of peripheral blood eosinophilia in subjects with airway hyperresponsiveness increases the risk to develop respiratory symptoms, such as persistent wheeze and dyspnea, whereas peripheral blood eosinophilia is not associated with the development of chronic cough and/or phlegm, and bronchitis episodes.
- ◆ Skin test positivity in the past decreases the risk to develop respiratory symptoms in the future, although the way in which skin test positivity has been determined may have influenced the results.



**Figure 2.** Schematic representation of the possible role of eosinophils in respiratory symptoms and airway hyperresponsiveness

*With regard to lung function development (Table 2):*

Subjects with AHR have more severe actual airways obstruction (pre-challenge level of FEV<sub>1</sub> % predicted and FEV<sub>1</sub> % VC), and have had a lower maximally attained level of FEV<sub>1</sub> between the ages 20 and 25, and a faster preceding decline in FEV<sub>1</sub> from age 25 onwards, than subjects without AHR. The higher risk for AHR with a lower level of FEV<sub>1</sub> % VC is significantly stronger in symptomatic than asymptomatic subjects.

◆ Symptomatic subjects with AHR (Sy+AHR+) have a lower actual level of FEV<sub>1</sub> % predicted and FEV<sub>1</sub> % VC than asymptomatic subjects without AHR (Sy-AHR-). The level of lung function is similar in subjects without AHR whether they had symptoms (Sy+AHR-) or not (Sy-AHR-). The maximally attained level of FEV<sub>1</sub> between the ages 20 and 25 and the preceding decline in FEV<sub>1</sub> from age 25 onwards are not associated with the presence of respiratory symptoms.

*With regard to airway wall inflammation:*

◆ In our study, there was no difference in airway inflammation between subjects without respiratory symptoms and AHR (Sy-AHR-) and subjects with the single or coinciding presence of respiratory symptoms and AHR (Sy-AHR+, Sy+AHR-, Sy+AHR+). Heterogeneity of characteristics within the four groups under study, including smoking habits and atopy, may have increased the variability in airway inflammation. Therefore, we cannot exclude the presence of an inflammatory process in the airways.

◆ Individuals with peripheral blood eosinophilia have higher numbers of eosinophils in the airway wall.

◆ Epithelial changes, as determined by various cytokeratins and integrins, were neither observed between subjects with and without respiratory symptoms and AHR, nor between subjects with different smoking habits.

**Table 2. Summary of associations of various atopy-related factors and lung function parameters with the single or coinciding presence of respiratory symptoms (Sy) and airway hyperresponsiveness (AHR)**

	Respiratory symptom (Sy) and airway hyperresponsiveness (AHR) status		
	Sy+AHR+	Sy+AHR-	Sy-AHR+
Peripheral blood eosinophilia	+	?	+
Skin test positivity	++	?	nsa
Serum total IgE level > 100 kU/L	nsa	?	nsa
Lower actual level of FEV <sub>1</sub> % predicted	++	nsa	0
Lower actual level of FEV <sub>1</sub> % VC	+	nsa	+
A lower maximally attained level of FEV <sub>1</sub> % pred. between the ages 20 and 25	+	nsa	+
A faster preceding decline in FEV <sub>1</sub> from age 25 onwards	++	nsa	+

The group of Sy-AHR- subjects was taken as reference category.

+ positive significant association,  
 ++ a significantly stronger positive association than +  
 nsa no significant association  
 ? association was not determined in this thesis

## Perspectives

Individuals with peripheral blood eosinophilia are more likely to have airway hyperresponsiveness (AHR) than individuals without peripheral blood eosinophilia (Chapter 3). Whether peripheral blood eosinophilia and AHR coincidentally result from the same stimulus or whether peripheral blood eosinophilia is involved causing AHR, remains unclear. Further longitudinal analyses using the Vlagtwedde-Vlaardingen data can be used to investigate whether peripheral blood eosinophilia precedes the development of AHR, and whether an increase in the number of peripheral blood eosinophils is associated with the development of AHR or more severe airway hyperresponsiveness. New studies may gain more information on the specific stimulus that attracts the eosinophils from the bone marrow into the peripheral circulation, and possibly causes AHR at the same time. Further, in addition to determining the number of eosinophils in the peripheral blood, studying the specific characteristics of the eosinophils may give further insight in these processes. These characteristics may include the level of expression of cellular adhesion molecules (CD11b, CD18 and VLA-4) and the affinity for their appropriate endothelial receptors, and the autocrine role of eosinophils in enhancing their own

survival period (IL-3, IL-5, GM-CSF) (9).

An inflammatory process in the airways and airway remodeling are considered to play an important role in asthma and COPD. We studied possible inflammatory changes in the airways of subjects with respiratory symptoms and AHR (Chapter 6a and 6b). Although no differences in inflammatory cell numbers and vascular adhesion molecules were observed, we cannot exclude the possibility that potential differences in the inflammatory process in the airways of subjects with and without respiratory symptoms and AHR remained obscured due to the heterogeneity of characteristics of the study subjects (such as smoking habits and atopy).

Cigarette smoking is one of the main factors in the development of respiratory symptoms in adults (Chapter 4), especially due to the high prevalence (almost 50%) of smoking in the population (Table 1, Chapter 4). A clear dose-response relationship was observed between the risk to develop respiratory symptoms and the number of cigarettes smoked at the first of two successive surveys (Table 2, Chapter 4). The use of more specific information on the changes in smoking habits between the two surveys in the analyses, makes it possible to assess the effect of quitting smoking compared with continued smoking in the future, as well as the effects of a reduction or increase in the number of cigarettes per day on the change in symptom status. Further, it can be investigated whether these effects are different for the various smoking categories (i.e. cigarettes, cigars and/or pipe or amount of tobacco smoked per day), and if there is a moment when quitting smoking or reducing the number of cigarettes smoked per day has no advantageous effect anymore on respiratory symptoms.

In most of our analyses, the symptom and AHR status were based on a single measurement. However, there is a considerable variability in both characteristics, and more measurements may be necessary to characterize the subjects more precisely. Information of the Vlagtwedde-Vlaardingen Study from 1967 to 1987 showed that 54% of all subjects were never hyperresponsive, 27% were hyperresponsive at one but not all surveys, and 19% were hyperresponsive at all surveys (13). The analysis described in this thesis showed that 46% of the subjects never reported respiratory symptoms, 48% reported symptoms at at least one but not all surveys, and only a small proportion of subjects (6%) reported symptoms at each survey (Table 4, Chapter 3). The risk of AHR for subjects with peripheral blood eosinophilia appeared to be mainly present in subjects with intermittent respiratory symptoms in the past and not in subjects who were never or persistently symptomatic. This suggests that new studies on the development of respiratory symptoms should especially focus on subjects with variable AHR and symptom status, since symptom and AHR status in the other groups hardly change. It is important to determine the risk factors for the development of AHR and respiratory symptoms in this group with intermittent symptoms, but even more important are the risk factors responsible for the development of a continued presence or absence of respiratory symptoms and AHR. Studies on the factors responsible for the continuous presence or absence of respiratory symptoms and AHR during adult life, may provide

information on the characteristics of subjects who are not at risk to develop respiratory symptoms and whose respiratory symptoms and AHR will not remit.

In conclusion, the atopy-related factors peripheral blood eosinophilia, skin test positivity, and high serum total IgE levels, and the various measures of lung function development, including the maximally attained level of FEV<sub>1</sub> between the ages 20 and 25, and the decline in FEV<sub>1</sub> from age 25 onwards, the actual level of FEV<sub>1</sub> % predicted and FEV<sub>1</sub> % VC, relate differently to airway hyperresponsiveness in the absence or presence of respiratory symptoms. Further, biopsy studies (or studies using bronchoalveolar lavage or induced sputum to collect information on the airway lumen) including more subjects in each group may reveal more information on a possible underlying inflammatory process. The use of multiple measurements of respiratory symptoms, AHR, and smoking habits may provide more specific information on the subjects most likely to develop continuous respiratory symptoms.

## References

1. de Vries, K., J.T. Goei, H. Booy-Noord, and N.G.M. Orie. 1962. Changes during 24 hours in the lung function and histamine hyperreactivity of the bronchial tree in asthmatic and bronchitic patients. *Int.Arch.Allergy* 20:93-101.
2. Eiser, N.M., K.F. Kerrebijn, and P.H. Quanjer. 1983. Guidelines for standardization of bronchial challenges with (nonspecific) bronchoconstricting agents. *Bull.Eur.Phyiopathol.Respir.* 19:495-514.
3. Chatham, M., E.R. Bleeker, P.L. Smith, R.R. Rosenthal, P. Mason, and P.S. Norman. 1982. A comparison of histamine, methacholine, and exercise airway reactivity in normal and asthmatic subjects. *Am.Rev.Respir.Dis.* 126:235-240.
4. Toelle, B.G., J.K. Peat, C.M. Salome, J. Crane, D. McMillan, J. Dermand, W. D'Souza, and A.J. Woolcock. 1994. Comparison of two epidemiological protocols for measuring airway responsiveness and allergic sensitivity in adults. *Eur.Respir.J.* 7:1798-1804.
5. Rijcken, B., J.P. Schouten, S.T. Weiss, A.F. Meinesz, K. de Vries, and R. van der Lende. 1989. The distribution of bronchial responsiveness to histamine in symptomatic and asymptomatic subjects; a population based analysis of various indices of responsiveness. *Am.Rev.Respir.Dis.* 140:615-623.
6. Anonymous. 1985. NHLBI Workshop Summaries: Summary and recommendations of a workshop on the investigative use of fiberoptic bronchoscopy and bronchoalveolar lavage in asthmatics. *Am.Rev.Respir.Dis.* 132:180-182.
7. Xu, X., B. Rijcken, J.P. Schouten, and S.T. Weiss. 1997. Airways responsiveness and development and remission of chronic respiratory symptoms in adults. *Lancet* 350:1431-1434.
8. Weller, P.F. 1997. Human eosinophils. *J.Allergy Clin.Immunol.* 100:283-287.
9. Rothenberg, M.E. 1998. Eosinophilia. *N.Engl.J.Med.* 338:1592-1600.
10. Daniels, S.E., S. Bhattacharya, A. James, N.I. Leaves, A. Young, M.R. Hill, J.A. Faux, G.F. Ryan, S.P. le, G.M. Lathrop, A.W. Musk, and W.O. Cookson. 1996. A genome-wide search for quantitative trait loci underlying asthma. *Nature* 383:247-250.
11. Postma, D.S. and H.A. Kerstjens. 1998. Characteristics of airway hyperresponsiveness in asthma and chronic obstructive pulmonary disease. *Am.J.Respir.Crit.Care Med.* 158:S187-92.
12. Paré, P.D. and T.R. Bai. 1996. Airway wall remodelling in chronic obstructive pulmonary disease. *Eur.Respir.Rev.* 6:259-263.
13. Rijcken, B., J.P. Schouten, S.T. Weiss, B. Rosner, K. de Vries, and R. van der Lende. 1993. Long-term variability of bronchial responsiveness to histamine in a random population sample of adults. *Am.Rev.Respir.Dis.* 148:944-949.



